0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 9
Printed in U.S.A.

Formation of Insulin-Producing Cells from Pancreatic Acinar AR42J Cells by Hepatocyte Growth Factor*

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ABSTRACT

Pancreatic AR42J cells are derived from acinar cells and express both exocrine and neuroendocrine properties. We have recently shown that these cells convert into insulin-producing cells *in vitro* after treatment with activin A and betacellulin. Here, we investigated the effect of hepatocyte growth factor (HGF) in those cells. When AR42J cells were incubated with HGF, DNA synthesis was attenuated, and the amylase content was reduced in a concentration-dependent manner. HGF-treated cells extended processes, but bundle formation was not observed using an antibody against tubulin. Reverse transcription-PCR analysis revealed that messenger RNAs for both insulin and pancreatic polypeptide (PP) were expressed in HGF-treated, but not naive, AR42J cells. Immunocytochemical analysis indicated that approximately 3% of the HGF-treated cells were stained with antiinsulin antibody, and some were also stained with anti-PP antibody. When AR42J cells were exposed to a combination of activin A and HGF, cells extended longer processes, and over 10% of them were stained with antiinsulin antibody. In these cells, messenger RNAs for insulin, PP, glucose transporter 2, and glucokinase, but not those for glucagon or somatostatin, were expressed. A subclone of AR42J cells, AR42J-B13, was obtained. Most of the AR42J-B13 cells converted to insulin-producing cells after the incubation with activin A and HGF. Insulin secretion was augmented by tolbutamide, depolarizing concentrations of potassium, carbachol, and glucagon-like peptide-1 in these cells. These results indicate that HGF reduces the acinar cell-like property of AR42J cells and converts them into insulin-producing cells. The effect of HGF was markedly enhanced by activin A. (*Endocrinology* 137: 3969–3976, 1996)

HEPATOCYTE growth factor (HGF) is a heterodimeric protein consisting of a 62-kDa α-chain and a 34/32-kDa β-chain. HGF was originally recognized as a growth factor involved in liver regeneration (1) and was purified from rat platelets (2) and the serum of patients with hepatic failure (3, 4). HGF was later proven identical to scatter factor, which dissociates MDCK cells (5). It is now recognized that HGF elicits diverse effects in various types of cells, particularly those of epithelial and endothelial origin (see Refs. 6 and 7 for review). These include mitogenic action, tumor suppression, motogenic action, and morphogenic and angiogenic effects. Principally, HGF acts as a paracrine factor produced by cells of mesenchymal origin and acts on epithelial cells. HGF affects the differentiation of pancreatic endocrine cells (8, 9). The formation of islet-like cell clusters in 6-day cultures of human fetal pancreatic tissue is stimulated 2- to 3-fold, and the insulin content of the cultures is increased by HGF. Furthermore, culture with HGF for 7 days leads to a 3-fold increase in *reg* gene expression, a molecular marker associated with regeneration of the pancreas.

AR42J cells are derived from a chemically induced rat pancreatic acinar carcinoma and are widely used to study the regulation of exocrine secretion (10). Logsdon *et al.* (11) reported that AR42J cells differentiate in response to dexamethasone, and amylase content as well as the number of zymogen granules increased. AR42J cells also possess some of the properties of neuroendocrine cells. For example, these cells have an electrically excitable membrane (12) and express synaptophysin (13), a marker of neuronal cells. Upon stimulation with activin A, AR42J cells differentiate into neuron-like cells (14). Thus, activin-treated cells extend neurite-like processes that contain the cytoskeletal architecture typical of neurites. Moreover, activin-treated cells express messenger RNA (mRNA) for the α_1-subunit of the β-cell/neuroendocrine-type voltage-dependent calcium channel (15) and ATP-sensitive potassium channel as well as the sulfonyl urea receptor (14). Given that pancreatic endocrine cells express several marker proteins common to those of neurons, exposure to activin A may provide a model system with which to study the conversion of pancreatic precursors to neuroendocrine cells. Consistent with this idea, we have shown recently that upon exposure to activin A and betacellulin, AR42J cells differentiate into cells secreting insulin (16). Collectively, AR42J cells provide a unique model system with which to study the formation of pancreatic endocrine cells *in vitro*. In the present study, we investigated the effect of HGF in amphicrine AR42J cells. The results indicated that HGF can convert AR42J cells into insulin-secreting cells.

Received January 21, 1996.

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* This work was supported by a Grant-in-Aid from the Ministry of Education, Science, and Culture of Japan and a grant from the Pancreatic Research Foundation of Japan.

Materials and Methods

Materials

Recombinant human HGF was provided by Prof. T. Nakamura of Osaka University (Osaka, Japan). Glucagon-like peptide-1-(7–37) amide (GLP-1) was purchased from Peptide Institute (Osaka, Japan). Recombinant human activin A (17) was provided by Dr. Y. Eto of Central Research Laboratory, Ajinomoto (Kawasaki, Japan). [^{3}H]Thymidine was

TABLE 1. Sequences of PCR primers

Gene	Sense primer	Antisense primer	Size of product (bp)	Nucleotide no.	GenBank accession no.
Insulin (I and II)	TGCCCAGGCTTTTGTCAAACAGCACCTT	CTCCAGTGCCAAGGTCTGAA	187	4244–4430 1177–1862	J00747 J00748
Glucagon	GTGGCTGGATTGTTTGTAATGCTGCTG	CGGTTCCTCTTGGTGTTCATCAAC	236	59–132 89–250	K02809 K02810
PP	TGAACAGAGGGCTCAATACGAAAC	AGACAGAAGGGAGGCTACAAATCC	214	2198–2692	M18207
GK	AAGGGAACAACATCGTAGGA	CATTGGCGGTCTTCATAGTA	136	47–96 1–80	M24947 M24948
GLUT2	TTAGCAACTGGGTCTGCAAT	GGTGTAGTCCTACACTCATG	343	1424–1766	J01345
Somatostatin	ACCCCAGACTCCGTCAGTTTCTG	TTCTTGCAGCCGCTTTGCGCGTTCCC	238	624–1482	J00786 J00787
β-Actin	CGTAAAGACCTCTATGCCAA	AGCCATGCCAAATGTCTCAT	349	2748–3220	J00691

obtained from DuPont-New England Nuclear (Boston, MA). Tolbutamide and carbachol were obtained from Sigma Chemical Co. (St. Louis, MO).

Cell culture

AR42J cells (10) and AR42J-B13 cells, a subclone of AR42J cells responsive to HGF, were cultured in DMEM containing 20 mM HEPES-NaOH (pH 7.4), 5 mM $NaHCO_3$, penicillin, streptomycin, and 10% FCS. AR42J-B13 cells were obtained from subclones of AR42J cells by measuring the ability to convert to insulin-positive cells in response to HGF. As commercially available FCS contains various amounts of activin A depending upon the lot, we checked the bioactivity of activin (18) in various lots and used activin-free FCS (Flow Laboratory, North Ryde, Australia). To measure amylase content, cells were plated at a density of 2×10^5 cells/well in 24-well plates.

Measurement of DNA synthesis

DNA synthesis was assessed by measuring [^{3}H]thymidine incorporation into trichloroacetic acid-precipitable materials. Cells cultured in 24-well plates were incubated in the presence or absence of HGF. Thereafter, 0.5 μCi/ml [^{3}H]thymidine was added to each well 4 h before termination of the reaction. [^{3}H]Thymidine incorporation was measured as previously described (19).

Measurement of amylase content

To measure amylase content, the medium was removed, and the cells were gently washed twice with PBS at 4 C. The cells were then scraped off into 1 ml of the buffer containing 50 mM NaH_2PO_4 and 50 mM NaCl (pH 6.9) and sonicated with a probe-type sonicator for 30 sec at 4 C. Amylase activity was then measured according to the method of Bernfeld (20). One amylase unit is defined as the amount of enzyme that hydrolyzes starch into 1 mg maltose during a 5-min reaction at 37 C. The protein concentration was measured as described by Bradford (21), using BSA as the standard. Statistical analysis was performed by Student's *t* test.

Antibodies

An affinity-purified rabbit polyclonal antibody against chicken β-tubulin was a gift from Prof. Kuniaki Takata of the Institute for Molecular and Cellular Regulation, Gunma University. Antihuman salivary amylase monoclonal antibody was purchased from Sigma. Polyclonal antibodies against porcine insulin and glucagon were provided by Prof. K. Wakabayashi of the Institute for Molecular and Cellular Regulation, Gunma University, and polyclonal antibovine pancreatic polypeptide (PP) was obtained from Dr. T. Takeuchi of the Institute for Molecular and Cellular Regulation. Other commercially available antibodies were indocarbocyanine-conjugated donkey antirabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), indocarbocyanine-conjugated donkey antiguinea pig IgG (Cappel, Turnhout, Belgium), and tetramethylrhodamine isothianate-conjugated goat antimouse IgG (Cappel).

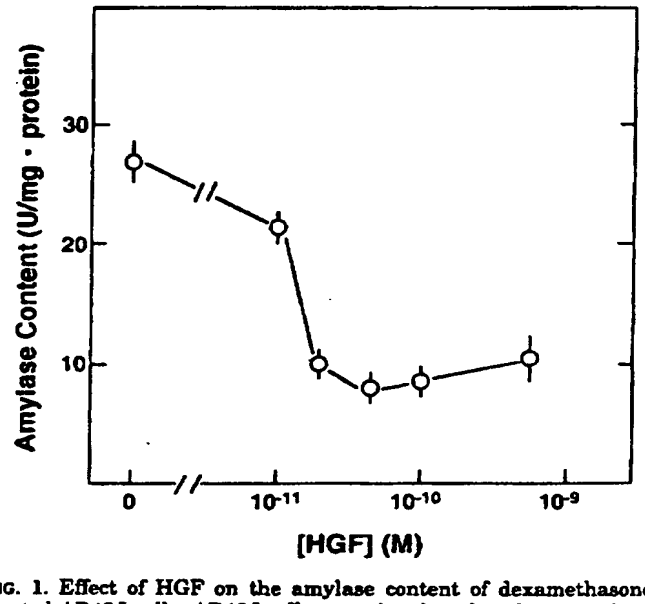


FIG. 1. Effect of HGF on the amylase content of dexamethasone-treated AR42J cells. AR42J cells were incubated with 10 nM dexamethasone and various concentrations of HGF for 2 days. The amylase content was then measured. Values are the mean ± SE for four experiments. The amylase content in dexamethasone-untreated cells was 6.93 ± 0.59 U/mg·protein.

Immunofluorescence microscopy

Cells were cultured on noncoated glass coverslips. The cells were fixed with 3% paraformaldehyde in PBS, solubilized with 0.1% (vol/vol) Triton X-100 in PBS for 5 min, and incubated sequentially with Blocking Ace (Morinaga, Tokyo, Japan), first and second antibodies. The cells were examined using a Zeiss Axiophoto microscope (New York, NY). For measurement of insulin-positive cells, incubation was performed in quadruplicate, and 500 cells were counted in each dish. Experiments were repeated at least three times.

Analysis of mRNA by reverse transcription-PCR (RT-PCR)

mRNA was extracted using a Quick Prep kit (Pharmacia, Piscataway, NJ). mRNA obtained from islets was used as a control template, and mRNA samples were digested with deoxyribonuclease to remove any contaminating genomic DNA. First strand complementary DNA was synthesized using the preamplification system for first strand complementary DNA synthesis kit (Life Technologies, Grand Island, NY). To confirm the absence of genomic DNA, samples were prepared without reverse transcriptase. The oligonucleotide primers used are listed in

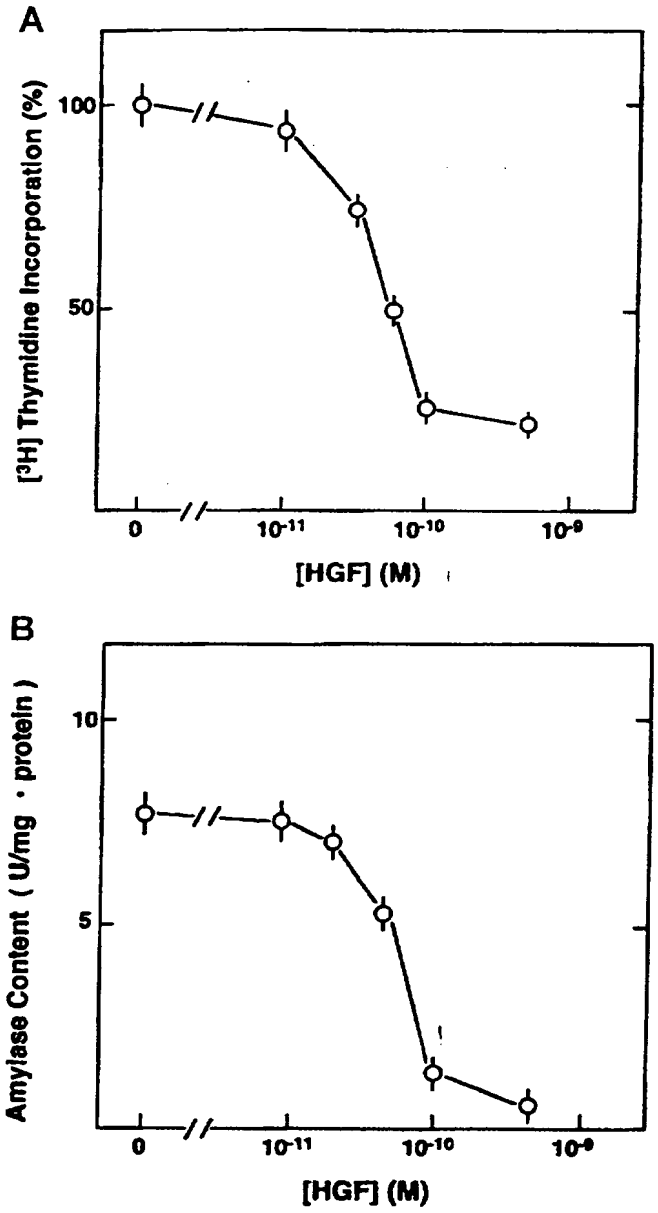


FIG. 2. Effects of HGF on DNA synthesis and the amylase content in AR42J cells. AR42J cells were incubated for 48 h with various concentrations of HGF, and DNA synthesis (A) and amylase content (B) were measured. DNA synthesis in the absence of HGF was 152,539 ± 5,875 dpm/well. After 48 h of incubation with 500 pM HGF, the cell number was 42.0 ± 24% of the control value. Values are the mean ± SE for four experiments.

Table 1. The reactions proceeded in a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, CT) in the presence of [α-^{32}P]deoxy-CTP under the following conditions: for insulin, glucokinase, and glucose transporter-2 (GLUT2), denaturation at 94 C for 1 min and annealing and extension at 65 C for 2 min; and for glucagon, PP, and β-actin, denaturation at 94 C for 30 sec, annealing at 60 C for 30 sec, and extension at 72 C for 45 sec. The number of cycles was 25, except for glucokinase and β-actin (23 and 20 cycles, respectively). The products were separated on a 5% polyacrylamide gel, which was dried and exposed to x-ray film.

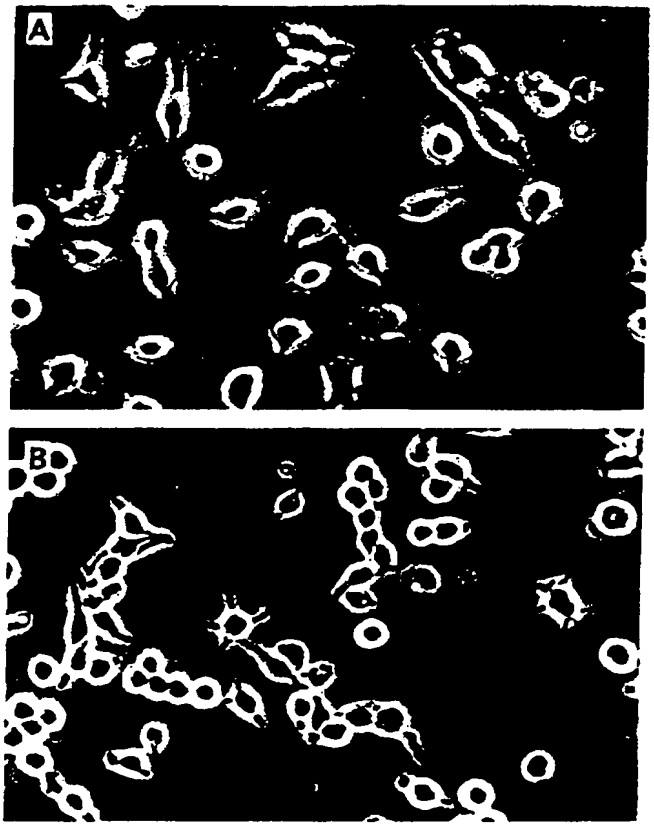


FIG. 3. Morphology of HGF-treated AR42J cells. Cells were incubated for 10 days with 100 pM HGF, and the morphology of the cell was examined (A and B). A, Bipolar cells; B, cells extending multiple processes.

Measurement of cytoplasmic free calcium

Cells were grown on a glass coverslips. The cells were loaded with the calcium-sensitive fluorescent dye fura-2 (23) by incubating them with 2 μM fura-2/acetoxymethylester for 30 min at room temperature. The fura-2-loaded cells were examined under a fluorescence microscope, and the fluorescence from a single cell was monitored as previously described (14).

Measurement of insulin secretion

AR42J-B13 cells were seeded at a density of 10^5 cell/well in a 24-well plate. The cells were incubated for 2 days with 100 pM HGF and 2 nM activin A. After washing with Krebs-Ringer bicarbonate buffer containing 5.5 mM glucose and 0.1% BSA, the cells were incubated with various stimulators for 1 h. Insulin released into the medium was measured as described previously (16). Statistical analysis was performed using Student's *t* test.

Results

Effect of HGF on dexamethasone-induced differentiation of AR42J cells

In AR42J cells, glucocorticoids induce differentiation into acinar-like cells. Thus, dexamethasone increases the number of zymogen granules, the steady state level of mRNA for amylase, and the content of the enzyme (11). We initially examined whether HGF modified the differentiation of AR42J cells induced by dexamethasone. Incubating the cells with dexamethasone for 48 h increased amylase content

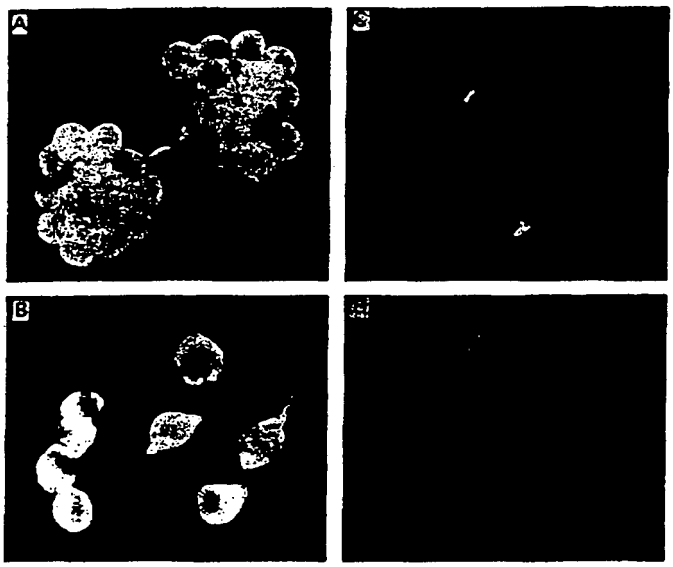


FIG. 4. Changes in the intracellular distribution of amylase in HGF-treated cells. Cells were incubated with 100 pM HGF for 0 (A), 2 (B), 5 (C), or 8 (D) days, and amylase was stained with antiamylase antibody. Note that six cells were in the field in C and D.

3-fold. When HGF was added together with dexamethasone, the effect of dexamethasone was attenuated by HGF (Fig. 1). The inhibitory effect of HGF was dose dependent; inhibition was maximal at 50 pM HGF. In the presence of 50 pM HGF, the amylase content was nearly identical to that in naive cells. Therefore, HGF almost completely abolished the effect of dexamethasone.

Effect of HGF on DNA synthesis in AR42J cells

The above results suggested that HGF attenuated dexamethasone-induced differentiation into acinar-like cells. We then examined whether HGF by itself modifies the characteristics of AR42J cells. As shown in Fig. 2A, HGF reduced DNA synthesis in AR42J cells. At a concentration of 500 pM, HGF almost completely blocked DNA synthesis. HGF also blocked the increase in cell number (data not shown). Figure 2B depicts the effect of HGF on the amylase content of the cells. HGF reduced the amylase content of the cells in a dose-dependent manner, and in the presence of 500 pM HGF, the amylase activity disappeared. Similar results were obtained in cells incubated for 5 days (data not shown). These results suggest that HGF attenuated the growth and induced differentiation in a direction distinct from that induced by dexamethasone.

Morphological changes induced by HGF

We then examined the changes in morphology of AR42J cells induced by HGF. When cells were treated with 100 pM HGF, they extended processes within 48 h. These gradually elongated in the presence of HGF (Fig. 3A). Most of the HGF-treated cells were bipolar, but some individual cells extended three or more processes (Fig. 3B). Figure 4 demonstrates the changes in immunofluorescence staining of amylase in HGF-treated cells. In naive AR42J cells, amylase

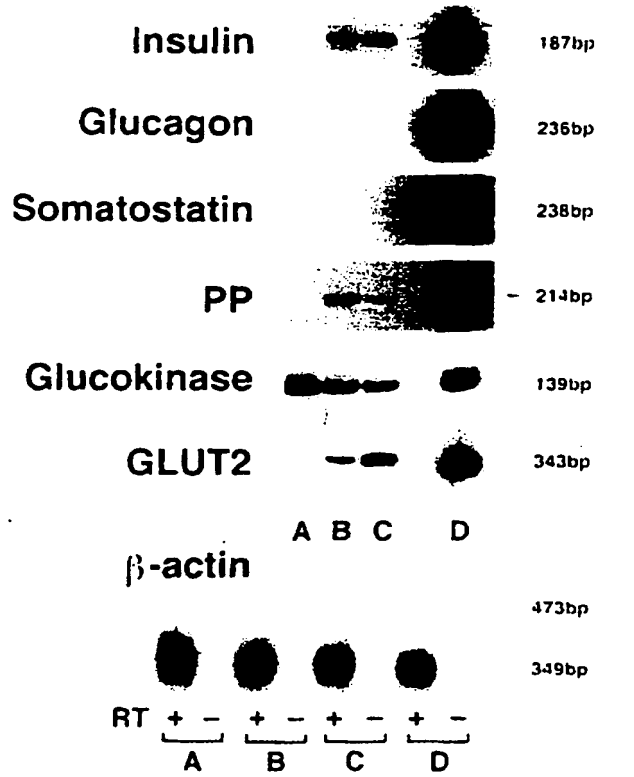
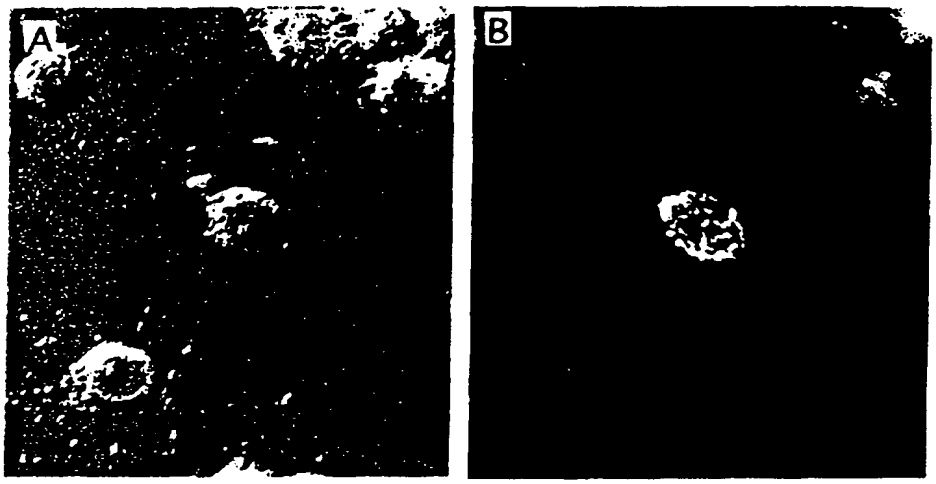


FIG. 5. Expression of mRNA in HGF-treated cells. mRNA was extracted from cells incubated for 5 days with (B and C) or without (A) 100 pM HGF in the presence (C) or absence (B) of 2 nM activin A. Rat pancreatic islet mRNA (D) was obtained as a positive control. Insulin, glucagon, PP, somatostatin, glucokinase, GLUT2, and β-actin mRNA expressions were analyzed by RT-PCR. Primers for β-actin span one small intron, and contaminated genomic DNA, if present, should be detected at a position of 472 bp. RT, Treatment with reverse transcriptase.

was diffusely distributed in the cytoplasm (Fig. 4A). On the second day of exposure to HGF, the amylase content decreased slightly (Fig. 4B), and on the fifth day, it decreased markedly (Fig. 4C). On the eighth day, there was virtually no staining of amylase (Fig. 4D). To characterize the cytoskeletal organization in cytoplasmic processes induced by HGF, we studied the participation of tubulin in HGF-induced processes. Naive AR42J cells contained tubulin in the cytoplasm. In HGF-treated cells, tublin was distributed in both the cytoplasm of the cell body and processes. Unlike the cells treated with activin A (14), the bundle-like structure in the process, which is a typical cytoskeletal structure of neurons (14), was not thick compared with that of activin-treated AR42J cells (14).

Effect of HGF on the expression of islet hormones

We then examined by means of RT-PCR whether HGF induced the expression of mRNA for various islet hormones. In HGF-treated cells, mRNAs for insulin and PP were detected, whereas mRNA for glucagon or somatostatin was undetectable (Fig. 5). Glucokinase and GLUT2 mRNAs were also detected. In naive AR42J cells, mRNAs for the three



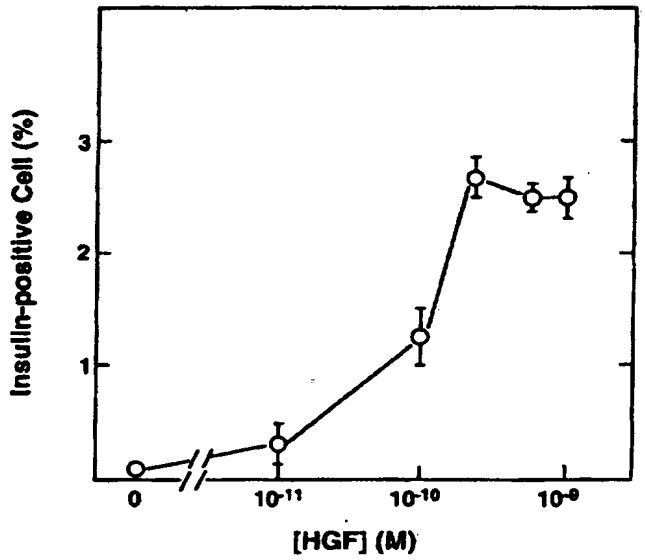


FIG. 6. Effect of HGF on the induction of insulin-positive cells. Cells were incubated for 5 days with 100 pM HGF, then stained with antiinsulin antibody (B). A, Nomarski image of the same area. C, Cells were incubated for 5 days with various concentrations of HGF, and the number of insulin-positive cells was counted. Values are the mean ± SE for four experiments.

hormones were undetectable, whereas that for glucokinase was detected. Immunocytochemical analysis revealed that insulin-positive cells were found in HGF-treated cells, but the number of insulin-positive cells was small. Insulin-positive cells were round (Fig. 6, A and B). Immunoreactivity disappeared upon preabsorption of the antibody with insulin (data not shown). Figure 6C shows the dose-response relationship for the HGF effect on the induction of insulin-positive cells. Nearly 3% of the cells became insulin positive, and tolbutamide did not increase the cytoplasmic Ca^{2+} levels in insulin-positive cells, which was subsequently confirmed by immunocytochemistry (data not shown).

Effect of the combination of HGF and activin A

Our study indicated that activin A converts AR42J cells into neuron-like cells (14). We, therefore, examined the effect of the combination of HGF and activin A. As shown in Fig. 7A, AR42J cells incubated with activin A and HGF extended long processes, and growth cone-like structures appeared. Immunocytochemically, cells with elongated processes were stained with an antiinsulin antibody (Fig. 7, B and C). Immunoreactive insulin accumulated in the terminals of the processes. Immunoreactive PP was also detected in some cells incubated with HGF and activin A (data not shown). PP-positive cells were round and smaller than the insulin-positive cells. RT-PCR revealed the expression of mRNAs for insulin, PP, GLUT2, and glucokinase, but not that for glucagon or somatostatin (Fig. 5). The mRNA level of GLUT2 in cells treated with HGF and activin A was higher than that in cells treated with HGF alone. In the presence of HGF and activin A, almost 10% of the cells were insulin positive.

Insulin secretion from AR42J-B13 cells

These results indicate that HGF converted a portion of AR42J cells into insulin-secreting cells. We, therefore, ob-

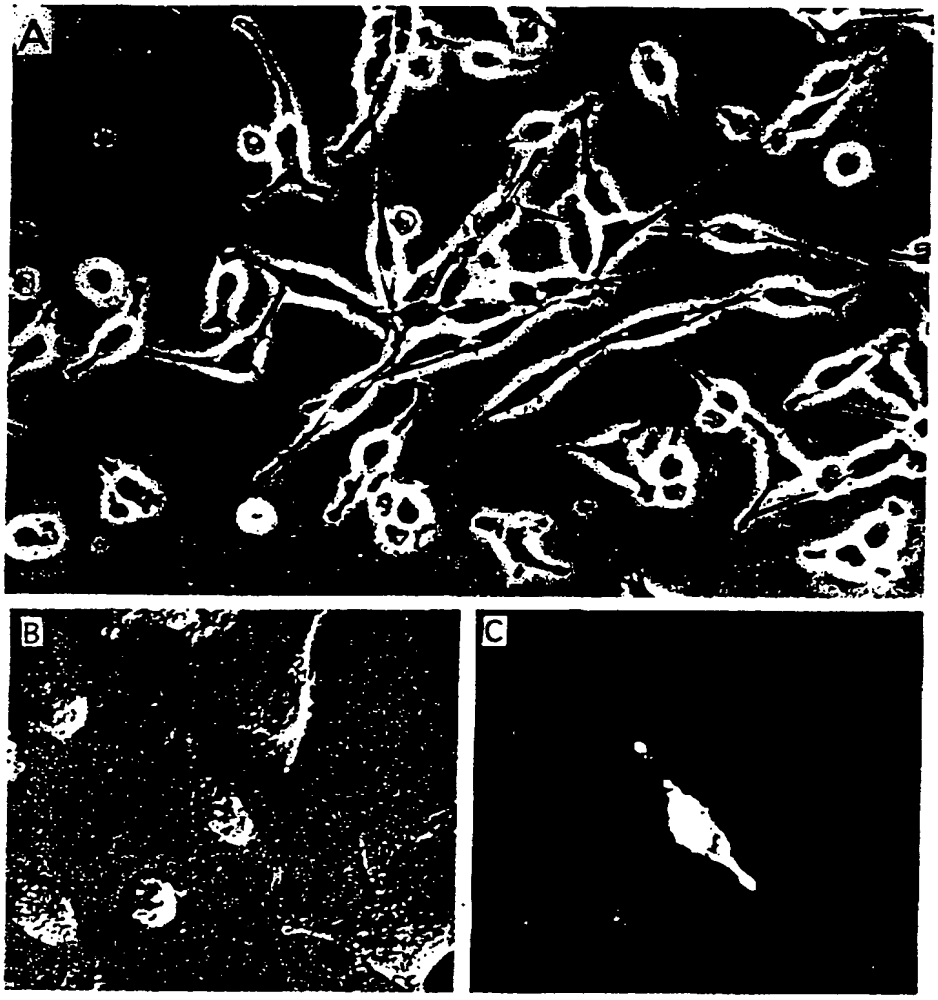


FIG. 7. Morphology of AR42J cells treated with HGF and activin A. Cells were incubated for 7 days with 2 nM activin A and 100 pM HGF, and the morphology of the cells was determined (A). Cells treated with HGF and activin A were stained with antiinsulin antibody (C). B, Nomarski image of the same area.

TABLE 2. Number of insulin-positive cells after treatment with HGF in the presence and absence of activin A

Treatment	Insulin-positive cells
None	0
HGF	19.4 ± 5.3
HGF + activin A	88.4 ± 7.8

AR42J-B13 cells were incubated for 5 days with 100 pM HGF in the presence and absence of 2 nM activin A. Insulin-positive cells were counted by immunocytochemistry. Values are the mean ± SE for four experiments.

tained a subclone of AR42J cells responsive to HGF, AR42J-B13. Approximately 20% of the AR42J-B13 cells were stained with antiinsulin antibody after exposure to 100 pM HGF (Table 2). In the presence of activin A and HGF, nearly 90% of the cells became insulin positive. As shown in Fig. 8, the depolarizing concentration of potassium induced a 2-fold increase in the secretion of immunoreactive insulin in AR42J-B13 cells exposed to HGF and activin A. Likewise, tolbutamide, carbachol, and glucagon-like peptide-1 increased insulin secretion significantly, whereas a high concentration of glucose had no effect.

Discussion

Pancreatic endocrine cells are thought to arise from precursor cells in the pancreatic duct (24, 25). Despite extensive studies, the process by which endocrine cells are formed from ductal precursors is poorly understood. Based on observations of transgenic mice expressing interferon-γ in pancreatic β-cells, Gu *et al.* (26) postulated that ductal cells first convert to cells expressing the properties of both ductal and acinar cells, which then convert to cells expressing both acinar and endocrine properties (acinar/endocrine cells). The latter type of precursor cells may eventually differentiate into endocrine cells. The AR42J cells used in this study resemble in many respects the acinar/endocrine cells found in regenerating pancreas described by Gu *et al.* (26). AR42J cells possess properties characteristic of both acinar and neuroendocrine cells (10, 14). They also differentiate into both acinar-like (11) and endocrine cells (16). As shown in Fig. 5, AR42J cells express glucokinase mRNA. This is surprising, but may be explained by the amphicrine nature of these cells. Using AR42J cells as a model system, we investigated the effect of HGF on the growth and differentiation of these cells. The results demonstrate three important aspects of the actions of

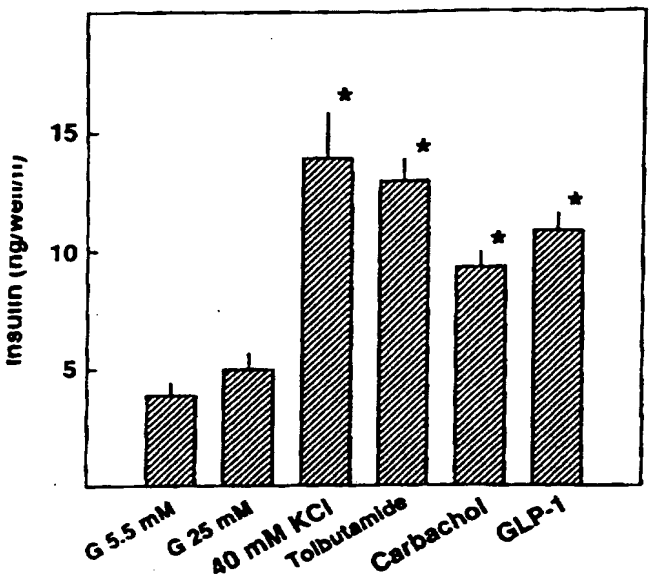


FIG. 8. Insulin secretion from AR42J-B13 cells treated with HGF and activin A. AR42J-B13 cells were incubated for 3 days with 100 pM HGF and 2 nM activin A. Cells were then incubated for 1 h in Krebs-Ringer bicarbonate buffer containing 40 mM KCl, 10 μM tolbutamide, 100 μM carbachol, 1 nM GLP-1, or 25 mM glucose. Note that the glucose concentration was 5.5 mM, except for stimulation with 25 mM glucose. Insulin released into the medium was measured. Values are the mean ± SE for four experiments. *, $P < 0.05$ *vs.* none.

HGF in these cells. First, HGF reduces the properties of exocrine cells and blocks the differentiation of AR42J cells to acinar-like cells induced by dexamethasone. Thus, HGF attenuates the dexamethasone-induced elevation of the amylase content. Secondly, HGF alone also reduced the amylase content of AR42J cells, terminated DNA synthesis, and elongated processes. These effects of HGF are in some respects similar to those induced by activin A (14). However, the actions of these two factors are distinct in some respects. For example, the morphological changes induced by these two factors are not identical, and unlike activin A, cells exposed to HGF do not express the cytoskeletal organization typical of neurons (27). Also, cytoplasmic Ca^{2+} responses to tolbutamide are absent in HGF-treated cells, suggesting that the sulfonyl urea receptor and/or ATP-sensitive potassium channel are deficient. The third aspect of the effect of HGF is that it promotes conversion into insulin-producing cells. Again, this effect of HGF is not elicited by activin A. In fact, activin A, which has no effect on the formation of insulin-producing cells, greatly potentiates the action of HGF. Therefore, the modes of actions of HGF and activin A are different, and the two factors act coordinately to promote the formation of insulin-producing cells. It should be mentioned that the dose-response relationships for the HGF-induced decrease in amylase content are different in the presence and absence of dexamethasone. This may be due to the difference in the number of HGF receptors in these two conditions. Alternately, the intracellular signaling systems activated by HGF are different in the two conditions.

The present results are in agreement with those reported by Otonkoski *et al.* (8), who showed that HGF augments the formation of islet-like cell clusters in cultured human fetal pancreatic tissue. They evaluated the effect of HGF in mixtures of cells including endocrine precursor cells. It was difficult to identify which type of cells was actually affected by HGF. Likewise, it was difficult to determine, in a strict sense, whether HGF converted the precursor cells to insulin-producing cells or HGF increased the content of insulin in cells already committed to become insulin-producing cells. Our results extend theirs, insofar as we found that HGF acts directly on endocrine precursor-like cells, which do not express islet hormones, and converts them into insulin-secreting cells. An alternate possibility is that a few cells convert to insulin-positive cells on their own and then are stimulated to proliferate by HGF. This possibility seems unlikely, because in both AR42J and AR42J-B13 cells, HGF inhibits proliferation. The effects of HGF on AR42J cells described here are similar to those induced by betacellulin. It is of interest to understand how these two factors induce similar effects because they act on distinct receptor systems. AR42J may be a suitable cell system with which to study the mechanisms of action of betacellulin and HGF on the differentiation of pancreatic endocrine cells.

AR42J-B13 cells pretreated with a combination of HGF and activin A synthesize and secrete immunoreactive insulin. In addition, they respond to secretagogues such as tolbutamide, carbachol, and GLP-1. Although these cells express mRNA for glucokinase and GLUT2, they do not respond to the elevation of ambient glucose. The exact reason for the unresponsiveness to glucose is not clear at present, but the results suggest that the operation of the glucose-sensing system is inadequate in these cells. Given the fact that glucokinase together with GLUT2 constitute the glucose-sensing mechanism in β-cells, it is possible that the protein level of either of two molecules is not enough to function as a glucose sensor.

This study demonstrated the coordinate actions of activin A and HGF. As described previously (16), activin A commits AR42J cells to differentiate into PP-producing cells. When activin A and HGF were added simultaneously to AR42J-B13 cells, most of them converted to insulin-producing cells. Whether these two factors act coordinately *in vivo* is not clear. However, it is notable that these two factors may be expressed in fetal pancreas. Activin A is expressed in endocrine precursor cells in rat pancreatic anlage (28) and in endocrine precursor cells in regenerating rat islets (Furukawa, M., and I. Kojima, unpublished observation). In addition, HGF is expressed in non-β-cells of rat pancreatic islets (29). HGF may also be supplied by mesenchymal cells. It is, therefore, an interesting possibility that these two factors play a role in the formation of endocrine cells *in vivo*. In this regard, mice lacking HGF (30, 31) and the HGF receptor (32) have been developed by homologous recombination. These mice, however, do not have abnormal pancreatic endocrine cells. The results, however, do not totally exclude the involvement of HGF in the formation of endocrine cells in the pancreas. As described previously, betacellulin has the same effect as HGF (16). Targeting of HGF or its receptor might be rescued by the alternative pathway of the redundant regulatory system. In any event, the *in vivo* roles of these factors should be examined experimentally by various approaches.

In summary, HGF and activin A coordinately convert amphicrine AR42J cells into insulin-producing cells. HGF may act as a differentiation factor in the endocrine pancreas.

Acknowledgments

The authors thank Prof. T. Nakamura of Osaka University and Dr. Y. Eto of Ajinomoto, Inc., for the generous gifts of HGF and activin A, respectively. We thank Kiyomi Ohgi for secretarial assistance during the preparation of the manuscript.

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